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[54] **ENZYME IMMUNOASSAYS USING IMMOBILIZED REAGENTS IN A FLOWING STREAM**

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[58] **Field of Search** ..... 435/4, 7, 288, 291, 435/14; 204/195 B; 23/230 B; 422/68, 63, 67, 71, 81, 82, 101; 424/12

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[57] **ABSTRACT**

This invention relates to a method and apparatus for the rapid quantitative determination of an antigen contained in aqueous biochemical samples, for example, blood. The method comprises a series of sequentially arranged stages through which a buffered stream flows. The sample, containing an unknown concentration of the antigen, is injected into the buffered flowing stream and contacts various reagents in the sequential series of stages. An initial solubilization stage comprises an immobilized antibody on a substrate which antibody is specific to the antigen in the sample. The immobilized antibody has been reacted previously to saturation with an enzyme-antigen complex, the antigen of the complex being the same as the antigen in the sample, and the complex is reversably bound to the immobilized antibody. The antigen in the sample and buffered stream flow through the solubilization stage and a competitive equilibrium reaction takes place between the complex bound to the antibody and the antigen in the sample, resulting in the release of a quantity of the enzyme-antigen complex into the flowing stream. The complex and the stream pass into a conversion stage wherein the complex reacts with an immobilized substrate, such as starch, to produce a measurable product which is a quantitative index of the complex released in the solubilization stage. The measurable product and the stream flow to a detection stage wherein the product is reacted, quantitatively measured and related to the concentration of the antigen in the original sample.

24 Claims, 3 Drawing Figures